Microspectrofluorometry on Fly Photoreceptors In Vivo. Dependence of Oxidative Metabolism on Light and Dark Adaptation

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PHOTOCHEMISTRY AND BIOCHEMISTRY OF BLOWFLY PHOTORECEPTOR MEMBRANES

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Spectrophotometric measurements performed with *isolated rhabdoms* and digitonin extracts prepared from *isolated rhabdomeric* membranes demonstrate that visual cells R1-6 of the blowfly (*Calliphora erythrocephala*) contain a rhodopsin with λ_{max} 490 nm and a slightly enhanced β-band at 340 nm. Irradiation of isolated rhabdoms with blue light converts rhodopsin into metarhodopsin (acid metarhodopsin λ_{max} 570 nm and/or alkaline metarhodopsin λ_{max} 380 nm) which, at 10°C, is thermally stable and can be reconverted by orange light into rhodopsin. Metarhodopsin formed in digitonin extracts decays *slowly* ($t_{1/2}$ 18 min, at 22°C) into opsin and all-*trans* retinal. In the *isolated retina* metarhodopsin as well as rhodopsin may exist in phosphorylated and non-phosphorylated states. Phosphorylation occurs after conversion of rhodopsin into metarhodopsin ($t_{1/2}$ 2 to 3 min at 25°C). Phosphorylated rhodopsin is formed by photoregeneration from phosphorylated metarhodopsin. The phosphorylated rhodopsin becomes rapidly dephosphorylated ($t_{1/2} < 20$ sec). A high percentage of adenylate cyclase activity and membrane bound cAMP phosphodiesterase activity present in the blowfly retina is associated with the rhabdomeric photoreceptor membrane. However, so far we have found no evidence that one of these enzyme activities is affected by the conversion of rhodopsin into metarhodopsin. Supported by the Deutsche Forschungsgemeinschaft.

MICROSPECTROFLUOROMETRY ON FLY PHOTORECEPTORS *IN VIVO*. DEPENDENCE OF OXIDATIVE METABOLISM ON LIGHT AND DARK ADAPTATION

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Visual pigment conversion was studied in relation with light induced oxidative metabolism in the photoreceptors of living, intact blowflies. The amount of conversion of rhodopsin to metarhodopsin caused by 0.25 sec flashes 494 nm light was quantitatively monitored by measuring the red (metarhodopsin) fluorescence. Conversions of $\gtrsim 10^4$ visual molecules in a dark adapted receptor induces an increase in green fluorescence (presumably from the mitochondrial flavoproteins) and a decrease in blue fluorescence (presumably from NADH). These effects are thought to be due to enhanced oxidation of the mitochondrial pigments. Saturation occurs when virtually all $\approx 10^8$ visual pigment molecules convert within the duration of the flash, i.e. at $\approx 10^{16}$ quanta $cm^{-2} s^{-1}$ (delivered by NPL10, 0.2 objective). We hypothesize that substantial rhodopsin conversion rapidly results in depletion of the intracellular energy buffer and therefore causes activation of the mitochondrial respiratory chain. The intensity dependence of this activation is directly proportional to the fraction of visual molecules existing in the rhodopsin station; the spectral dependence equals that of rhodopsin absorption. Flash induced activation depends on the preceding dark adaptation time in a way identical to that of the receptor potential; half time of sensitivity recovery is 15–30 sec. Anoxia results within several seconds in a decrease in metabolic energy. Hence continuous mitochondrial activity is a vital requirement for photoreceptor function.

MICROSPECTROFLUOROMETRY ON FLY PHOTORECEPTORS *IN VIVO*. AUTOFLUORESCENCE OF VISUAL AND MITOCHONDRIAL PIGMENTS

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Microspectrofluorometry has been performed on living, intact blowflies *Calliphora erythrocephala*, mutant chalky. Three pigments, each with a distinct emission can be distinguished. (I) Red emission